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(54) **ANTITISSUE FACTOR ANTIBODY COMPLEX**

(57)Abstract:
PROBLEM TO BE SOLVED: To provide a novel antihuman tissue factor antibody complex. SOLUTION: This anti-tissue factor antibody complex is a complex of an antihuman tissue factor antibody with a chemotherapeutic agent and a toxin or a vascularization inhibitor and useful as an active ingredient of antitumor medicinal compositions.

CLAIMS

[Claim(s)]
[Claim 1] Complex which connects the fragment and chemotherapic drug which have the antibody (anti-tissue-factor antibody) or antigen binding capacity to a tissue factor, and changes.
[Claim 2] Complex which connects the fragment and toxin which have the antibody or antigen binding capacity to a tissue factor by the linker, and changes.
[Claim 3] Complex which connects the fragment and blood vessel new inhibitor which have the antibody or antigen binding capacity to a tissue factor, and changes.
[Claim 4] the aforementioned tissue factor—array number: -- complex given in any 1 term of the claims 1-3 which have the amino acid sequence shown in 1
[Claim 5] Complex given in any 1 term of the claims 1-4 whose aforementioned anti-tissue-factor antibodies are monoclonal antibodies.

[Claim 6] Complex given in any 1 term of the claims 1-4 whose aforementioned anti-tissue-factor antibodies are reconstruction man antibodies.
[Claim 7] the aforementioned reconstruction man antibody is a man antibody -- coming out -- complex given in any 1 term of some claims 1-4
[Claim 8] the fragment which has the antigen binding capacity of the aforementioned anti-tissue-factor antibody -- Fab, F(ab ') 2, scFv, or sc (Fv)2 it is -- complex given in any 1 term of claims 1-7
[Claim 9] Complex given in any 1 term of claims 1-8 whose aforementioned chemotherapic drug is an antitumor agent.
[Claim 10] The aforementioned antitumor agent Mel FARAN (Melphalan) and cisplatin (Cis-platinum), Carboplatin (Carboplatin) Mitomycin C (Mitomycin C), Adriamycin (Adriamycin; Doxorubicin), A daunorubicin (Daunorubicin) and bleomycin (Bleomycin), A neocarzinostatin (Neocarzinostatin) and a methotrexate (Methotrexate), 5-fluoro uridine (5-Fluorouridine), 5-fluoro-2'-deoxyuridine (5-Fluoro-2'-deoxyuridine), Cytosine arabinoside (Cytosine arabinoside), an aminopterin (Aminopterin), vincristine (Vincristine), paclitaxel (Paclitaxel), and DOSETA cheating on the fare (Docetaxel) Or vindesine (Vindesine) it is -- complex according to claim 9
[Claim 11] Complex given in any 1 term of claims 1-8 whose aforementioned chemotherapic drug is a cytokine.
[Claim 12] Complex according to claim 11 whose aforementioned cytokine is interleukin-2, tumor necrosis factor alpha (TNFalpha), or interferon (IFN).
[Claim 13] The aforementioned toxin A diphtheria-toxin A chain (Diphtheria toxin A chain), The Pseudomonas endotoxin (Pseudomonas endotoxin), A lysine A chain (Ricin A chain) and a non-sugar chain lysine A chain (Deglycosylated ricin A chain), A A chain (A chain), a gelonin (Gelonin), and pork WIDO anti-virus protein (PAP-s; Pokeweed anti-viral protein from seeds), BURIOJIN (Briodin), saporin (Saporin), and peach RUJIN (Momordin), A MOMORU caulking (Momorcochin), dianthin 32 (Dianthin 32), Dianthin 30 (Dianthin 30) and MODESSHIN (Modeccin), Screw cumin (Viscumin) and BORUKESHIN (Volkesin), Dodecandrin (Dodecandrin), TORICHIN (Tritin), and RUFIN (Luffin) Or complex given in the claim 2 and any 1 term of 4-8 which are a TORIKO giraffe (Trichokirin).
[Claim 14] Complex given in the claim 1 which connects the fragment and chemotherapic drug which have an anti-tissue-factor antibody or antigen binding capacity by the linker, and changes, and any 1 term of 4-12.
[Claim 15] Complex which changes including the fragment which has the anti-tissue-factor antibody or antigen binding capacity which connected combination or the liposome which inner-**(ed), a micell, or porosity polymer for the chemotherapic drug by the linker.
[Claim 16] Complex which changes including the fragment which connects combination or the liposome which inner-**(ed), a micell, or porosity polymer for a blood vessel new inhibitor by the linker, and has an anti-tissue-factor antibody or antigen binding capacity.
[Claim 17] Complex which changes including the fragment which has the anti-tissue-factor antibody or antigen binding capacity which connected combination or the liposome which inner-**(ed), a micell, or porosity polymer for toxin by the linker.
[Claim 18] The aforementioned linker 3-(2-pyridyl dithiol) propionyl hydrazide, N-SUKUSHINIMIJIRU 3-(2-pyridyl dithio) propionate, LC-SUKUSHINIMIJIRU 3-(2-pyridyl dithio) propionate, Sulfo-LC-SUKUSHINIMIJIRU 3-(2-pyridyl dithio) propionate, N-SUKUSHINIMIJIRU 3-(2-pyridyl dithio) butyrate, SUKUSHINIMIJIRU oxy-carbonyl-alpha-(2-pyridyl dithio) toluene, LC-SUKUSHINIMIJIRU oxy-carbonyl-alpha-(2-pyridyl dithio) toluene, Sulfo-LC-SUKUSHINIMIJIRU oxy-carbonyl-alpha-(2-pyridyl dithio) toluene, SUKUSHINIMIJIRU-4-(p-maleimide phenyl) butyrate, sulfo-SUKUSHINIMIJIRU 4-(p-maleimide phenyl) butyrate, m-maleimide benzoyl-N-hydro KISHISUKUSHINIMIDO ester, Sulfo-m-maleimide benzoyl-N-hydro KISHISUKUSHINIMIDO ester, S-acetyl mercapto SUKUSHINIKKUANHIDORAIDO, dimethyl Complex given in any 1 term of claims 2-17 which is a 3 and 3-dithio screw PURORIONIMI date or a 2-imino thio lane.
[Claim 19] the aforementioned linker -- a peptide, the carboxymethyl (dextran CM) horse mackerel bottle biotin, a polyethylene glycol (PEG), a dextran, an amino dextran, a SHISU aconitic acid, glutamic-acid dihydrazide, or human serum albumin (HSA) it is -- complex given in any 1 term of claims 2-17
[Claim 20] Complex given in any 1 term of the claims 1, 4-12 which connect the fragment and chemotherapic drug which have an anti-tissue-factor antibody or antigen binding capacity by the middle base material, and change.
[Claim 21] claim 2- which connects the fragment and toxin which have an anti-tissue-factor antibody or antigen binding capacity by the middle base material, and changes -- complex given in any 1 term of 8, 13, and 15-17
[Claim 22] the aforementioned middle base material -- a peptide, a carboxymethyl dextran (CM), an avidin biotin, a polyethylene glycol (PEG), a dextran, an amino dextran, or human serum albumin (HAS) it is -- complex according to claim 20 or 21
[Claim 23] The medicine constituent which grows into any 1 term of claims 1-22 including the complex of a publication.
[Claim 24] The medicine constituent according to claim 23 which has antitumor action.
[Claim 25] The constituent for a diagnosis which grows into any 1 term of claims 1-23 including the complex of a publication.

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[The technical field to which invention belongs] this invention belongs to a medicinal field and relates to the complex which changes including an anti-tissue-factor antibody.
[0002]
[Description of the Prior Art] As a marker discovered as a marker which recognizes an endothelial cell widely till present by CD31, CD36, Ulex europaeus-lagglutinin (UEA-1), and the endothelial cell that was generally activated and was enlarged, von Willebrand factor (vWF), ICAM-1 (CD54), and E-selectin are known.
[0003] On the other hand, having discovered the specific antigen which has not been discovered in normal tissue is guessed by the endothelial cell (tumor vessel endothelial cell) which exists in a tumor in-house. E-9 antibody which recognizes various tumor vessel endothelial cells till present (Wang, J.M.et al., and Int.J.Cancer (1993) 54, 36), Anti-FB5 (endosialin) Antibody (Rettig, W.J.Pro.Natl.Sci.USA (1992) 89, 10832), H4/18 antibody (Cotran, R.S.et al., J.Exp.Med.(1986) 164, 661), Q BEND/10 antibody (Ramani, P.et al., Histopathology (1990) 17, 237), EN4/EN3 antibody (Schlingemann, R.O.et al., Amer.J.Pathol.(1991) 138, 1335), BMA120 antibody (Schlingemann, R.O.et al., Amer.J.Pathol.(1991) 138, 1335), EN7/44 antibody (Hagemeier, H.H.et al., and Int.J.Cancer (1986) 38, 481), PAL-E antibody (Schlingemann, R.O.et al., Amer.J.Pathol.(1991) 138, 1335), HEC-1 antibody (Gougos, A.et al., J.Immunol.(1988) 141, 1934), TEC4 and TEC11 (1994) Monoclonal antibodies, such as an antibody (Thorpe, P.E.et al., Am.Assoc.Cancer Res.(abstract) 35, 379), have been reported. However, the monoclonal antibody by which Kamiichi was carried out as a carrier of the medicine which makes a tumor vessel endothelial cell a target does not yet exist.
[0004] It is known for having played the role important for the start of blood coagulation, and a tissue factor is the disseminated intravascular coagulation syndrome (diffuse intravascular coagulation). It is considered one of the concerned important factors. On the other hand VEGF which is a vascularization factor (it Zucher(s)) S., et al., and Int.J.Cancer, 75, 780-786, and 1998 TGF-beta (Vrana., J.A., et al., Cancer Res., 56, 5063-5070, 1996), TNF-alpha (Zhang, Y, et al., J.Clin.Invest., 97, 2213-2224, 1996), It is reported that a tissue factor is guided to an endothelial cell by the cytokine of IL-1 grade. In recent years, a tissue factor is considered to be one of the factors in connection with the vascularization increasingly (Koomagi, R, Volm, M., and Int.J.Cancer, 79, 19-2, 1998).
[0005] Furthermore, in a good nature mammary gland tumor patient's tumor organization lumen formation vessel inner bark, the tissue-factor manifestation of the tumor vessel inner bark a breast cancer patient's cancer organization is checked to the tissue-factor manifestation having been negative in the immunity tissue-staining color (Contrino, J., et al., Nature Medicine, 2, 209-215, 1996). Moreover, it is solidification ability (procoagulant assy, PCA) from many human tumor cell stocks and tumors. It is made an index and tissue-factor activity is a report (Edwards, R.L., et al., Thrombosis and Haematosis, 69, 205-213, 1993). It is carried out and a tissue factor is considered [that it can use also as a cancer antigen and].
[0006] Therefore, it is thought that an anti-tissue-factor antibody may serve as a specific medicine carrier to the tumor which has discovered the tumor vessel inner bark or the tissue factor (WO 94/05328). However, an anti-tissue-factor antibody carries out an in TANA rise, a medicine is made to send into a tumor vessel endothelial cell as a medicine carrier, and it is not known whether it is possible to make the anti-tumor effect discover.
[0007]
[Problem(s) to be Solved by the Invention] this invention tends to introduce an antitumor agent etc. into a tumor cell or a tumor vessel endothelial cell using the internalization of an anti-tissue-factor antibody, and tends to offer the new means for checking proliferation of a tumor cell.
[0008]
[Means for Solving the Problem] In order that this invention persons may solve the above-mentioned technical problem, as a result of studying many things, an anti-human-tissue factor antibody and toxin complex show the protein synthesis prevention effect by low concentration extremely to the human-cancer cell which has discovered the tissue factor to cell surface, furthermore, an anti-tissue-factor antibody irrespective of the existence of the neutralization activity about the solidification ability of a tissue factor It succeeds in checking that the toxin complex shows the killer cell effect to the human-cancer cell which has discovered the tissue factor to cell surface. Consequently, the thing for which other matter in which the anti-tissue-factor antibody carried out the in TANA rise (Internalize) to the cell which discovers a tissue factor, and which it connected with this antibody on that occasion can be introduced into this matter, And the anti-tissue-factor antibody discovered that it was not based on the neutralization activity of a tissue factor, but the purpose could be attained, and completed this invention.
[0009] Therefore, this invention offers the complex which connects the fragment and chemotherapic drug which have the antibody (anti-tissue-factor antibody) or antigen binding capacity to a tissue factor, and changes. this invention offers the complex which connects the fragment and toxin which have an anti-tissue-factor antibody or antigen binding capacity again, and changes. this invention offers the complex which connects the fragment and chemotherapic drug which have the anti-tissue-factor antibody or antigen binding capacity combined with the human-tissue factor which becomes the array number 1 from the amino acid sequence of a publication again, or toxin, and changes.
[0010] this invention offers the complex which connects the fragment and chemotherapic drug which have the antibody or antigen binding capacity to a tissue factor, or toxin again, and changes. this invention offers the complex which connects the fragment and chemotherapic drug which have an anti-tissue-factor monoclonal

antibody or antigen binding capacity, or toxin again, and changes. this invention offers the complex which connects the fragment and chemotherapic drug which have antigen binding capacity, or toxin again, and changes.
[0011] this invention offers the complex which connects the fragment and chemotherapic drug which have a reconstruction Homo sapiens anti-tissue-factor antibody or antigen binding capacity, or toxin again, and changes. this invention offers the complex which connects the fragment and chemotherapic drug which have a reconstruction Homo sapiens antibody or antigen binding capacity, or toxin again, and changes. this invention is Fab which has an anti-tissue-factor antibody or its antigen avidity, F(ab ') 2, scFv, and 2 (scFv) again. The complex which connects a chemotherapic drug or toxin and changes is offered. this invention offers the complex which connects the fragment and antitumor agent which have the antibody or antigen binding capacity to a tissue factor again, and changes.
[0012] The fragment with which this invention has the antibody or antigen binding capacity to a tissue factor again, The merphalan (Melphalan) and cisplatin (Cis-platinum), Carboplatin (Carpoplatin), mitomycin C (Mitomycin C), Adriamycin (Adriamycin; Doxorubicin), A daunorubicin (Daunorubicin) and bleomycin (Bleomycin), A neocarzinostatin (Neocarzinostatin) and a methotrexate (Methotrexate), 5-fluoro uridine (5-Fluorouridine), 5-fluoro-2'-deoxyuridine (5-Fluoro-2'-deoxyuridine), Cytosine arabinoside (Cytosine arabinoside), an aminopterin (Aminopterin), vincristine (Vincristine), or vindesine (Vindesine) The complex which it comes to connect is offered.
[0013] this invention offers the complex which connects the fragment and cytokine which have the antibody or antigen binding capacity to a tissue factor again, and changes. this invention offers the complex which connects the fragment, and the interleukin-2 (IL-2), the tumor necrosis factor alpha (TNF-alpha) and interferon (IFN) which have the antibody or antigen binding capacity to a tissue factor again, and changes.
[0014] The fragment with which this invention has the antibody or antigen binding capacity to a tissue factor again, and a diphtheria-toxin A chain (Diphtheria toxin A chain), The Pseudomonas endotoxin (Pseudomonas endotoxin), A lysine A chain (Ricin A chain) and a non-sugar chain lysine A chain (Deglycosylated ricin A chain), A A chain (A chain), a gelonin (Gelonin), and pork WIDO anti-virus protein (PAP-s; Pokeweed anti-viral protein from seeds), BURIOJIN (Briodin), saporin (Saporin), peach RUJIN (Momordin), A MOMORU caulking (Momorcochin), dianthin 32 (Dianthin 32), Dianthin 30 (Dianthin 30) and MODESSHIN (Modeccin), Screw cumin (Viscumin), BORUKESHIN (Volkesin), dodecandrin (Dodecandrin), TORICHIN (Tritin), and RUFIN (Luffin) Or the complex which connects a TORIKO giraffe (Trichokirin) and changes is offered.
[0015] this invention offers the complex which connects the fragment and chemotherapic drug which have an anti-tissue-factor antibody or antigen binding capacity by the linker again, and changes. this invention offers the complex which connects the fragment and toxin which have an anti-tissue-factor antibody or antigen binding capacity by the linker again, and changes. this invention offers the complex which connects the fragment and chemotherapic drug which have the anti-tissue-factor antibody or antigen binding capacity combined with a human-tissue factor again, or toxin by the linker, and changes.
[0016] this invention offers the complex which connects the fragment and chemotherapic drug which have the antibody or antigen binding capacity to a tissue factor, or toxin by the linker again, and changes. this invention offers the complex which connects the fragment and chemotherapic drug which have an anti-tissue-factor monoclonal antibody or antigen binding capacity, or toxin by the linker again, and changes. this invention offers the complex which connects the fragment and chemotherapic drug which have an anti-tissue-factor antibody (IFO No.50382) or antigen binding capacity, or toxin by the linker again, and changes.
[0017] this invention offers the complex which connects the fragment and chemotherapic drug which have a reconstruction Homo sapiens anti-tissue-factor antibody or antigen binding capacity, or toxin by the linker again, and changes. this invention offers the complex which connects the fragment and chemotherapic drug which have a reconstruction Homo sapiens anti-tissue-factor antibody or antigen binding capacity, or toxin by the linker again, and changes. this invention offers the complex which connects Fab which has an anti-tissue-factor antibody or its antigen avidity, F(ab ') 2 or scFv, a chemotherapic drug, or toxin by the linker again, and changes. this invention offers the complex which connects the fragment and antitumor agent which have the antibody or antigen binding capacity to a tissue factor by the linker again, and changes.
[0018] The fragment with which this invention has the antibody or antigen binding capacity to a tissue factor again, The merphalan (Melphalan) and cisplatin (Cis-platinum), Carboplatin (Carboplatin), mitomycin C (Mitomycin C), Adriamycin (Adriamycin; Doxorubicin), A daunorubicin (Daunorubicin) and bleomycin (Bleomycin), A neocarzinostatin (Neocarzinostatin) and methotrexate (Methotrexate), 5-fluoro uridine (5-Fluorouridine), 5-fluoro-2'-deoxyuridine (5-Fluoro-2'-deoxyuridine), Cytosine arabinoside (Cytosine arabinoside), an aminopterin (Aminopterin), vincristine (Vincristine), or vindesine (Vindesine) The complex which it comes to connect by the linker is offered.
[0019] this invention offers the complex which connects the fragment and cytokine which have the antibody or antigen binding capacity to a tissue factor by the linker again, and changes. this invention offers the complex which connects the fragment, and the interleukin-2 (IL-2), the tumor necrosis factor alpha (TNF-alpha) and interferon (IFN) which have the antibody or antigen binding capacity to a tissue factor by the linker again, and changes.
[0020] The fragment with which this invention has the antibody or antigen binding capacity to a tissue factor again, and a diphtheria-toxin A chain (Diphtheria toxin A chain), The Pseudomonas endotoxin (Pseudomonas endotoxin), A lysine A chain (Ricin A chain) and a non-sugar chain lysine A chain (Deglycosylated ricin A chain), A A chain (A chain), a gelonin (Gelonin), and pork WIDO anti-virus protein (PAP-s; Pokeweed anti-viral protein from seeds), BURIOJIN (Briodin), saporin (Saporin), peach RUJIN (Momordin), A MOMORU caulking (Momorcochin), dianthin 32 (Dianthin 32), Dianthin 30 (Dianthin 30) and MODESSHIN (Modeccin), Screw cumin (Viscumin) and BORUKESHIN (Volkesin), Dodecandrin (Dodecandrin), TORICHIN (Tritin), and RUFIN (Luffin) Or

the complex which connects a TORIKO giraffe (Trichokirin) by the linker and changes is offered.
[0021] this invention offers the complex which connects the fragment and chemotherapic drug which have an anti-tissue-factor antibody or antigen binding capacity by the middle base material again, and changes. this invention offers the complex which connects the fragment and toxin which have an anti-tissue-factor antibody or antigen binding capacity by the middle base material again, and changes. this invention offers the complex which connects the fragment and chemotherapic drug which have the anti-tissue-factor antibody or antigen binding capacity combined with the human-tissue factor which becomes the array number 7 from the amino acid sequence of a publication again, or toxin by the middle base material, and changes.
[0022] this invention offers the complex which connects the fragment and chemotherapic drug which have an anti-tissue-factor monoclonal antibody or antigen binding capacity, or toxin by the middle base material again, and changes. this invention offers the complex which connects the fragment and chemotherapic drug which have a tissue-factor antibody or antigen binding capacity, or toxin by the middle base material again, and changes. this invention offers the complex which connects the fragment and chemotherapic drug which have a reconstruction Homo sapiens anti-tissue-factor antibody or antigen binding capacity, or toxin by the middle base material again, and changes.
[0023] this invention offers the complex which connects the fragment and chemotherapic drug which have a reconstruction Homo sapiens anti-tissue-factor antibody or antigen binding capacity, or toxin by the middle base material again, and changes. Fab in which this invention has an anti-tissue-factor antibody or its antigen avidity again, F(ab ') 2, scFv, or sc (Fv) -- two The complex which connects a chemotherapic drug or toxin by the middle base material, and changes is offered. this invention offers the complex which connects the fragment and antitumor agent which have the antibody or antigen binding capacity to a tissue factor by the middle base material again, and changes.
[0024] The fragment with which this invention has the antibody or antigen binding capacity to a tissue factor again, The merphalan (Melphalan) and cisplatin (Cis-platinum), Carboplatin (Carboplatin), mitomycin C (Mitomycin C), Adriamycin (Adriamycin; Doxorubicin), A daunorubicin (Daunorubicin) and bleomycin (Bleomycin), A neocarzinostatin (Neocarzinostatin) and methotrexate (Methotrexate), 5-fluoro uridine (5-Fluorouridine), 5-fluoro-2'-deoxyuridine (5-Fluoro-2'-deoxyuridine), Cytosine arabinoside (Cytosine arabinoside), An aminopterin (Aminopterin), vincristine (Vincristine), or vindesine (Vindesine) The complex which it comes to connect by the middle base material is offered.
[0025] this invention offers the complex which connects the fragment and cytokine which have the antibody or antigen binding capacity to a tissue factor by the middle base material again, and changes. this invention offers the complex which connects the fragment, and the interleukin-2 (IL-2), the tumor necrosis factor alpha (TNF-alpha) and interferon (IFN) which have the antibody or antigen binding capacity to a tissue factor by the middle base material again, and changes.
[0026] The fragment with which this invention has the antibody or antigen binding capacity to a tissue factor again, and a diphtheria-toxin A chain (Diphtheria toxin A chain), The Pseudomonas endotoxin (Pseudomonas endotoxin), A lysine A chain (Ricin A chain) and a non-sugar chain lysine A chain (Deglycosylated ricin A chain), A A chain (A chain), a gelonin (Gelonin), and pork WIDO anti-virus protein (PAP-s; Pokeweed anti-viral protein from seeds), BURIOJIN (Briodin), saporin (Saporin), peach RUJIN (Momordin), A MOMORU caulking (Momorcochin), dianthin 32 (Dianthin 32), Dianthin 30 (Dianthin 30) and MODESSHIN (Modeccin), Screw cumin (Viscumin) and BORUKESHIN (Volkesin), Dodecandrin (Dodecandrin), TORICHIN (Tritin), and RUFIN (Luffin) Or the complex which connects a TORIKO giraffe (Trichokirin) by the middle base material, and changes is offered.
[0027] this invention offers the medicine constituent which changes again including the complex indicated above. this invention offers the medicine constituent which has the antitumor action which changes again including the complex indicated above.
[0028]
[Embodiments of the Invention] The antigen to the anti-tissue-factor antibody used in this invention is already known. As an antigen for obtaining the anti-tissue-factor antibody used for this invention, the cell which has discovered the tissue factor, or the cell which made the tissue factor discover by transgenics is sufficient as Escherichia coli etc., and refining or half-refining tissue-factor protein is sufficient from such a cell. As such a cell, the various cell strains described above, for example in the term of the conventional technology can be used.
[0029] Although a polyclonal antibody or a monoclonal antibody is sufficient as the antibody used for this invention, especially its monoclonal antibody is desirable. A monoclonal antibody uses well-known technology fundamentally, and can create it as follows. That is, a tissue factor or a tissue-factor manifestation cell, for example, man vesical cancer origin cell J82 stock, is used as a sensitization antigen, immunity of this is carried out according to the usual immunity method, the immunocyte obtained is united with a well-known parent cell by the usual cell fusion method, and it can create by screening a monoclonal antibody forming cell by the usual screening procedure.
[0030] Especially as mammalian by which immunity is carried out with a sensitization antigen, although not limited, preferably [choosing in consideration of conformity with the parent cell which acts on cell fusion], and generally a mouse, a rat, a hamster, a rabbit, etc. are used. In order to carry out immunity of the sensitization antigen to an animal, it is carried out according to a well-known method. For example, it is carried out as the general method by injecting the inside of the peritoneal cavity of mammalian, or hypodermically with a sensitization antigen. It is desirable to carry out proper quantity mixture of the usual adjuvant, for example, Freund Freund's complete adjuvant, and to specifically, medicate mammalian with what diluted the sensitization antigen with PBS (Phosphate-Buffered Saline), the physiological saline, etc. in the suitable amount, and was suspended several times after emulsification by request, day by day [4 - 21]. Moreover, support suitable at the time of

sensitization antigen immunity can be used.
[0031] Thus, after checking that carry out immunity and desired antibody level rises in a blood serum, although an immunocyte is taken out from mammalian and cell fusion is given, ****** is mentioned especially as a desirable immunocyte. The myeloma cell of the mammalian as a parent cell of another side united with the aforementioned immunocyte Already well-known various cell strains (P3x63Ag8.653), P3 [for example,], (J.Immunol.123: 1548, 1978) p3-U1 (Current Topics in Micro-biology and Immunology81:1-7, 1978), NS-1 (Eur.J.Immunol.6:511-519, 1976), NPC-11 (Cell, 8:405-415, 1976), SPs 2/0 (Nature, 276:269-270, 1978), FO (J.Immunol.Meth.35:1-21, 1980), S194 (J.Exp.Med.148:313-323, 1978) and R210 (Nature, 277:131-133, 1979) etc. -- it is used suitably
[0032] The cell fusion of the aforementioned immunocyte and a myeloma cell can be fundamentally performed according to a well-known method (Milstein et al., Methods Enzymol.73:3-46, 1981), for example, Milstein's and others method etc. More specifically, the aforementioned cell fusion is carried out in the usual nutrition culture medium under existence of for example, a cell fusion accelerator. as a fusion accelerator -- for example, a polyethylene glycol (PEG) and a Sendai virus (HVJ) etc. -- it is used, and in order for a request to raise fusion efficiency further, addition use of the adjuvants, such as dimethyl sulfoxide, can also be carried out
[0033] As for the operating rate of an immunocyte and a myeloma cell, it is desirable to make an immunocyte into one to 10 times for example, to a myeloma cell. the usual culture medium used for RPMI1640 suitable culture medium for multiplication of the aforementioned myeloma cell strain, MEM culture medium, and this kind of other cell cultures as culture medium used for the aforementioned cell fusion, for example -- usable -- further -- fetal calf serum (FCS) etc. -- a blood serum water addition can also be used together
[0034] Cell fusion often mixes the specified quantity of the aforementioned immunocyte and a myeloma cell in the aforementioned culture medium, the PEG solution warmed at about 37 degrees C, for example, a with an average molecular weight of about 1000 to 6000 PEG solution, is usually beforehand added by 30 - 60% (w/v) of concentration, and the target syncytium (hybridoma) is formed by mixing. Then, the cell fusion which is not desirable is removable to growth of a hybridoma by repeating operation of adding serially, carrying out centrifugal [of the suitable culture medium], and removing a supernatant liquid.
[0035] The hybridoma concerned is chosen by cultivating with usual selective-culture liquid (culture medium containing a hypoxanthine, an aminopterin, and thymidine), for example, a HAT medium. The cultivation by the HAT medium concerned is usually continued several days to several weeks sufficient time for cells other than the hybridoma made into the purpose (non-syncytium) to become extinct. Subsequently, the usual limiting dilution is enforced and the screening and cloning of a hybridoma which produce the target antibody are performed.
[0036] Moreover, carry out immunity of the antigen to animals other than a man, and the above-mentioned hybridoma is obtained, and also sensitization can be carried out in the antigen protein or the antigen manifestation cell of the request of a man lymphocyte by in vitro, a sensitization B lymphocyte can be united with a man myeloma cell, U266 [for example,], and the man antibody of the request which has the avidity to a specific antigen or a specific antigen manifestation cell can also be obtained (refer to JP,1-59878,B). Furthermore, the transgenic animal which has the repertory of a man antibody gene may be medicated with an antigen or an antigen manifestation cell, and a desired man antibody may be acquired according to the above-mentioned method (refer to international patent application public presentation number WO 93-12227, WO 92-03918, WO 94-02602, WO 94-25585, WO 96-34096, WO 96-33735, and U.S. patent number US5545806).
[0037] Thus, the hybridoma which produces the monoclonal antibody produced can carry out subculture in the usual culture medium, and can carry out a mothball in liquid nitrogen. In order to acquire a monoclonal antibody from the hybridoma concerned, according to the usual method, cultivate the hybridoma concerned, mammalian with this and conformity is made to prescribe for the patient and increase the method of acquiring as the culture supernatant, or a hybridoma, and the method of acquiring as the ascites etc. is adopted. The former method is suitable for obtaining the antibody of a high grade, and, on the other hand, the latter method is suitable for mass production method of an antibody.
[0038] Carry out cloning of the antibody gene to this invention from an antibody forming cell, for example, a hybridoma, as a monoclonal antibody, and it includes in a suitable vector. The rearranged type antibody which introduced this into the host and was made to produce using gene modification technology can be used. (For example) Carl and A.K.Borrebaeck, Janes, W.Larrick, THERAPEUTIC MONOCLONAL ANTIBODIES, and Published in the United Kingdom by MACMILLAN PUBLISHERS LTD, 1990 references.
[0039] the immunocyte which specifically produces the hybridoma which produces the target antibody, and an antibody, for example, a sensitized lymphocyte etc., -- oncogene (oncogene) etc. -- mRNA which carries out the code of the variable region (V field) of an antibody is isolated from the cell which carried out immortalization The isolation of mRNA prepares all RNA by the well-known method (Chirgwin, J.M. et al., Biochemistry (1979)18, 5294-5299), for example, a guanidine ultracentrifugal method, the AGPC method (Chmczynski, P. and others (1987), 162, 156-159), etc., and prepares mRNA using mRNA Purification Kit (product made from Pharmacia) etc. Moreover, mRNA can be directly prepared by using QuickPrep mRNA Purification Kit (product made from Pharmacia).
[0040] cDNA of an antibody V field is compounded using reverse transcriptase from obtained mRNA. composition of cDNA -- AMV Reverse Transcriptase First-strand cDNA SynthesisKit etc. -- it can carry out by using moreover, the [perform / composition and amplification of cDNA] method using 5'-Ampli FINDER RACE Kit, and (product made from Clontech) PCR 5'-RACE (Frohman, M.A. et al. --) Proc.Natl.Acad.Sci.USA (1988) 85 and 8998-9002 ; Belyavsky and A. ** -- Nucleic Acids Res.(1989) 17 and 2919-2932 can be used The DNA fragment made into the purpose from the acquired PCR product is refined, and it connects with Vector DNA. Furthermore, it rearranges from this, a vector is created, it introduces into Escherichia coli etc., a colony is chosen, and a desired recombination vector is prepared. The base sequence of the target DNA is checked by the well-known method,

for example, the deoxy method.
[0041] If DNA which carries out the code of the V field of the target antibody is obtained, it will connect with DNA which carries out the code of the antibody constant region (C field) of a request of this, and this will be included in an expression vector. Or you may also include DNA which carries out the code of the V field of an antibody in the expression vector containing DNA of an antibody C field. In order to manufacture the antibody used by this invention, it includes in an expression vector so that an antibody gene may be discovered under control of manifestation regulatory region, for example, an enhancer/promotor, like the after-mentioned. Next, the transformation of the host cell can be carried out by this expression vector, and an antibody can be made to discover.
[0042] The manifestation of an antibody gene may include separately the heavy chain (H chain) or light chain (L chain) of an antibody in an expression vector, may include DNA which may be made to carry out joint transformation of the host, or carries out the code of an H chain and the L chain in a single expression vector, and may carry out the transformation of the host (international patent application public presentation number WO94/11523 reference). The recombined type antibody of a gene artificially changed for the purpose of reducing the heterologous-antigen nature to a man etc. in this invention, for example, a chimera, (Chimeric) An antibody, a man mold-ized (Humanized) antibody, or a hominization antibody can be used. These alteration antibodies can be manufactured using a known method.
[0043] A chimera antibody connects DNA which carries out the code of the antibody V field which is the above, and was made and obtained with DNA which carries out the code of the man antibody C field, includes this in an expression vector and is obtained by introducing into a host and making him produce (the Europe patent application public presentation number EP 125023, international patent application public presentation number WO95/14041 reference). A useful chimera antibody can be obtained to this invention using this known method.
[0044] A man mold-ized antibody is reconstruction (reshaped). It is also called a man antibody and is the complementarity determination field (CDR; complementarity determiningregion) of mammalians other than a man, for example, a mouse antibody. It transplants to CDR of a man antibody and the general transgenics technique is also known (the Europe patent application public presentation number EP 125023, international patent application public presentation number WO95/14041 reference).
[0045] It compounds by the PCR method from some oligonucleotides produced so that it might have the portion which overlaps at an end the DNA array specifically designed so that the framework field (FR; framework region) of a man antibody might be connected with CDR of a mouse antibody. Obtained DNA is connected with DNA which carries out the code of the man antibody C field, subsequently to an expression vector is incorporated, and it is obtained by making a host introduce and produce this (the Europe patent application public presentation number EP 239400, international patent application public presentation number WO95/14041 reference).
[0046] That in which FR of the man antibody connected through CDR forms an antigen binding site with good CDR is chosen. If needed, you may replace the amino acid of FR of V field of an antibody so that CDR of a reconstruction man antibody may form a suitable antigen binding site (Sato, K.et al., Cancer Res.(1993) 53, 851-856). A man antibody C field is used for a chimera antibody and a man mold-ized antibody. As a desirable man antibody C field, C gamma is mentioned, for example, 4 [Cgamma 1 or Cgamma 2 or Cgamma 3 or Cgamma] can be used. Moreover, in order to improve an antibody or the stability of the production, you may embellish a man antibody C field.
[0047] Since a chimera antibody consists of a V field of the mammalian antibody origin of those other than a man, and a C field of the man antibody origin, a man mold-ized antibody consists of CDR of the mammalian antibody origin of those other than a man, FR of the man antibody origin, and a C field and the antigenicity in the man inside of the body is falling, it is useful as an antibody used for this invention. The antibodies used by this invention may be an antibody fragment and an antibody ornamentation object, as long as it may be used suitable for this invention. For example, as an antibody fragment, Fab, F(ab') 2, Fv, or the single chain Fv (scFv) is mentioned. scFv has the structure with which Fv of an H chain and an L chain was made to connect by the suitable linker. Moreover, one or more amino acid residues of the amino acid sequence which constitutes an antibody are used as an antibody in this invention, and deal also in the antibody which received variation, substitution, a deletion, or insertion.
[0048] [whether in order to obtain these antibody fragments, an antibody is processed by the enzyme, for example, a papain, and the pepsin, and an antibody fragment is made to generate, and] Or after building the gene which carries out the code of these antibodies fragment and introducing this into an expression vector, It is made discovered by the suitable host cell (for example). Co, M.S.et al., and J. Immunol.(1994) 152, 2968-2976, Better, and M. & Horwitz and A.H.Methods in Enzymology (1989) 178, 476-496, and Academic Press, Inc., and Plueckthun, A.& Skerra and A.Methods in Enzymology (1989) 178, 476-496, and Academic Press, Inc., and Lamoyi, E., Methods in Enzymology (1989) 121,652-663, Rousseaux, J.et al., and Methods in Enzymology (1989) 121, 663-669, Bird, R.E.et al., TIBTECH (1991)9, and 132-137 Reference.
[0049] scFv is obtained by connecting the H chain V field and L chain V field of an antibody (the international patent application public presentation number WO 88-09344 and 95 to WO14041 reference). this scFv -- setting -- an H chain V field and an L chain V field -- a linker -- it is preferably connected through a peptide linker (Huston, J.S.et al., Proc.Natl.Acad.Sci.U.S.A.(1988) 85, 5879-5883) Although the H chain V field and L chain V field region in scFv were indicated as the above-mentioned antibody, they may be which the origin. As a peptide linker which connects V field, the arbitrary single strand peptides which consist of 12 to amino acid 19 residue, for example are used (refer to U.S. JP,5525491,B).
[0050] DNA which carries out the code of the scFv The H chain of the aforementioned antibody Or DNA which carries out the code of the H chain V field and an L chain The DNA portion which uses as mold DNA which carries

out the code of the L chain V field, and carries out the code of the amino acid sequence of the request of those arrays is amplified by the PCR method using the primer pair which specifies the ends. or subsequently It is obtained by amplifying DNA which furthermore carries out the code of the peptide linker portion, and its ends combining the primer pair it is prescribed that connects with an H chain and an L chain respectively.
[0051] Moreover, if DNA which once carries out the code of the scFv is produced, the host as for whom the transformation was done by the expression vector containing them and this expression vector can be obtained according to a conventional method. Moreover, according to a conventional method, scFv can be obtained using the host. The gene is acquired as mentioned above, it can be made discovered, and these antibodies fragment can be made to produce by the host. These antibody fragments are also included by the "antibody" as used in the field of this application patent claim. Since molecular weight is small compared with an antibody molecule, these antibody fragments are excellent in organization translatability in the living body, and useful as a molecule which has the same function as an antibody.
[0052] as an antibody ornamentation object -- polyethylene glycol (PEG) etc. -- the antibody combined with various molecules can also be used The ornamentation made by the antibody may be ornamentation by introducing chemical combination, and may be ornamentation made by the amino acid sequence of an antibody. These antibody ornamentation objects are also included by the "antibody" as used in the field of this application patent claim. In order to obtain such an antibody ornamentation object, it can obtain by embellishing to the obtained antibody. These methods are already established in this field.
[0053] It can be made discovered by the well-known method, and the antibody gene built as mentioned above can acquire an antibody. When using a mammals cell, it can be made discovered by the vector containing DNA or it which combined the poly A signal with useful promotor/enhancer used regularly, the antibody gene discovered, and 3' side lower stream of a river functionally. For example, as a promotor/an enhancer, a promotor/enhancer (human cytomegalovirus immediate early promoter/enhancer) can be mentioned in the first half of a man cytomegalovirus.
[0054] moreover -- in addition -- as the promotor/enhancer which can be used for the antibody manifestation used by this invention -- a virus promotor / enhancers, such as a retrovirus, a polyol virus, an adenovirus, and simian virus 40 (simian virus 40), and human elongation factor 1alpha (HEF1alpha) etc. -- what is necessary is just to use the promotor/enhancer of the mammals cell origin for example, the case where Mulligan's and others method (Nature (1979) 277, 108), and a HEF1alpha promotor / enhancer are used when using a simian virus 40 promotor / enhancer -- Mizushima ** -- method (Nucleic Acids Res.(1990) 18, 5322) If it follows, it can carry out easily.
[0055] It can be made to be able to join together functionally and the signal sequence for the useful promotor used regularly and antibody secretion and the antibody gene made to discover can be made to discover in the case of Escherichia coli. For example, a lacZ promotor and an araB promotor can be mentioned as a promotor. What is necessary is just to follow Better's and others method (Science (1988) 240, 1041-1043), when using a lacZ promotor and using Ward's and others method (Nature (1098) 341,544-546; FASEB J.(1992) 6, 2422-2427), and an araB promotor.
[0056] What is necessary is just to use a pelB signal sequence (Lei, S.P.et al J.Bacteriol.(1987) 169, 4379) as a signal sequence for antibody secretion, when making peri PURAZUMU of Escherichia coli produce. It is fold (refold) appropriately about the structure of antibody after separating the antibody produced by peri PURAZUMU. It is used by carrying out (see the international patent application public presentation number WO 96-30394 and Japanese patent application public notice JP,7-93879,B).
[0057] As the duplicate origin, the thing of the origins, such as a simian virus 40 polyoma virus, an adenovirus, and a bovine papilloma virus (BPV), can be used. Furthermore, an expression vector is an aminoglycoside transferase (APH) as a selective marker at a host cell system because of gene copy number amplification. A gene, a thymidine kinase (TK) gene, and Escherichia coli xanthin guanine HOSUHORIBOSHIRU transferase (Ecogpt) A gene, a dihydrofolic acid reductase (dhfr) gene, etc. can be included. Production systems arbitrary for manufacture of the antibody used by this invention can be used. The production system for antibody manufacture is in vitro and in vivo. There is a production system. in The production system which uses the production system which uses an eukaryotic cell, and a prokaryotic cell as a production system of vitro is mentioned.
[0058] When using an eukaryotic cell, there is a production system using an animal cell, a plant cell, and a fungus cell. As an animal cell, (1) mammals cell (J.Exp.Med.(1995) 108, 945), for example, CHO COS, myeloma, and BHK (baby hamster kidney), HeLa, Vero, (2) amphibian cells (Valle, et al., Nature (1981) 291, 358-340), for example, a platanna oocyte, or (3) insect cells 9, for example, sf, sf21, and Tn5 are known. CHO Especially as a cell, it is CHO which suffered a loss in the DHFR gene. dhfr-CHO (Proc.Natl.Acad.Sci.USA (1980) 77, 4216-4220) and CHO K-1 which are a cell (Proc.Natl.Acad.Sci.USA (1968) 60, 1275) It can be used suitably.
[0059] As a plant cell, it is Nicotiana tabacum. What is necessary is to know the cell of the origin and just to carry out the callus culture of this. As a fungus cell, yeast (Saccharomyces cerevisiae), for example, a Saccharomyces (Saccharomyces) group, for example, Saccharomyces cerevisiae, and mold (Aspergillus niger), for example, an ASUPERUGIUSU (Aspergillus) group, for example, an Aspergillus niger etc., are known. When using a prokaryotic cell, there is a production system using a bacterial cell. As a bacterial cell, Escherichia coli (E. coli) and the Bacillus subtilis are known.
[0060] An antibody is obtained by introducing the target antibody gene into these cells by the transformation, and cultivating the cell by which the transformation was carried out by in vitro. Cultivation is performed according to a well-known method. For example, DMEM, MEM, and RPMI1640 IMDM can be used as culture medium. that time -- fetal calf serum (FCS) etc. -- a blood serum water addition can also be used together and non-blood serum cultivation may be carried out Moreover, it is in vivo by moving the cell which introduced the antibody gene to the peritoneal cavity of an animal etc. You may produce an antibody.

[0061] On the other hand, it is in vivo. The production system which uses the production system which uses an animal, and vegetation as a production system is mentioned. An antibody gene is introduced into these animals or vegetation, and in the body of an animal or vegetation, an antibody is made to produce and it collects. When using an animal, there is a production system using a mammals animal and an insect. A goat, a pig, a sheep, a mouse, and a cow can be used as a mammals animal (Vicki Glaser, SPECTRUM Biotechnology Applications, 1993). Moreover, a transgenic animal can be used when using a mammals animal.
[0062] For example, the protein produced peculiar in milk like goat beta casein in an antibody gene is inserted in the middle of the gene which carries out a code, and it prepares as a fused gene. The DNA fragment containing the fused gene in which the antibody gene was inserted is poured in to the germ of a goat, and this germ is introduced to a female goat. A desired antibody is obtained from the milk which the transgenic goat produced from the goat which has received the germ, or its descendant produces. In order to make the amount of milk containing the antibody of the request produced from a transgenic goat increase, you may use hormone for a transgenic goat suitably. (Ebert, KM.et al., Bio/Technology (1994) 12, 699-702) .
[0063] Moreover, as an insect, a silkworm can be used, for example. When using a silkworm, Baculoviridae which inserted the target antibody gene is infected with a silkworm, and a desired antibody is obtained from the body fluid of this silkworm (Susumu, M.et al., Nature (1985) 315, 592-594). Tobacco can be used when using vegetation furthermore. When using tobacco, the target antibody gene is inserted in the vector 530 for a vegetable manifestation, for example, pMON, and it is this vector Agrobacterium tumefaciens It introduces into bacteria [like]. It is tobacco, for example, Nicotiana tabacum, about these bacteria. You make it infected and a desired antibody is obtained from the leaf of this tobacco (Julian, K-C.Ma et al., Eur.J.Immunol.(1994) 24,131-138).
[0064] They are in vitro or in vivo as mentioned above. When producing an antibody by the production system, DNA which carries out the code of the H chain or L chain of an antibody may be separately included in an expression vector, DNA which may be made to carry out joint transformation of the host, or carries out the code of an H chain and the L chain may be included in a single expression vector, and the transformation of the host may be carried out (94 to international patent application public presentation number WO11523 reference). As the introductory method of the expression vector to a host, a well-known method (Virology (1973) 52, 456-467), for example, a calcium phosphate method, the electroporation method (EMBO J.(1982) 1, 841-845), etc. are used.
[0065] It can dissociate from the inside and outside of a cell, and a host, and the antibody produced and discovered as mentioned above can be refined even to homogeneity. Separation of the antibody used by this invention and refining are not limited at all that what is necessary is just to use the separation and the refining method which are used by refining of the usual protein. For example, an antibody can be separated and refined if chromatography columns, such as affinity chromatography, a filter, an ultrafiltration, a salting-out, dialysis, etc. are chosen and combined suitably (Antibodies : A Laboratory Manual.Ed Harlow and David Lane, Cold Spring Harbor Laboratory, 1988).
[0066] As a column used for affinity chromatography, a protein A column and a protein G column are mentioned, for example. Hyper D, POROS, Sepharose F.F., etc. are mentioned as support used for a protein A column (Pharmacia). As chromatographies other than affinity chromatography For example, ion exchange chromatography, a hydrophobic chromatography, Gel filtration, reversed phase chromatography, An adsorption chromatography etc. is mentioned (). [Strategies forProtein Purification and Characterization:A Laboratory Course Manual.Ed] Daniel R.Marshak et al., Cold Spring Harbor Laboratory Press, 1996.
[0067] These chromatographies can be performed using liquid phase chromatographies, such as a liquid phase chromatography, for example, HPLC, and FPLC. Density measurement of the obtained antibody can be performed by measurement of an absorbance, or the enzyme joint immunity adsorption assay (enzyme-linked immunosorbent assay; ELISA). That is, when based on measurement of an absorbance, after diluting the obtained antibody with PBS suitably, the absorbance of 280nm is measured. For example, what is necessary is just to compute ml as 1.35OD(s) in 1mg /in the case of a man antibody.
[0068] Moreover, ELISA When depending, it can measure as follows. namely, the goat diluted [ml] with M-fold [0.1] carbonic acid buffer solution (pH 9.6) in 1microg /-- anti- -- man IgG(product made from TAGO)100microl is added to 96 hole plate (product made from Nunc), it incubates at 4 degrees C overnight, and an antibody is ******(ed) Man IgG(product made from CAPPEL)100microl of known concentration is added as the sample containing the antibody or antibody used by this invention diluted suitably after blocking, or a concentration reference standard, and it incubates at a room temperature for 1 hour.
[0069] the alkaline phosphatase indicator diluted 5000 times after washing -- anti- – man IgG(product made from BIO SOURCE)100microl is added, and it incubates at a room temperature for 1 hour After washing, a substrate solution is added, the absorbance in 405nm is measured after incubation using MICROPLATE READER Model 3550 (product made from Bio-Rad), and the concentration of the target antibody is computed. Moreover, BIAcore (product made from Pharmacia) can be used for the density measurement of an antibody.
[0070] Evaluation of the antigen avidity of the antibody used by this invention can use the usually learned method, for example, ELISA, EIA (enzyme immunoassay), RIA (radiation immunoassay method), or a fluorescent antibody technique (Antibodies : A Laboratory Manual.Ed Harlow and David Lane, Cold Spring Harbor Laboratory, 1988). BIAcore (product made from Pharmacia) can be used for activity evaluation of the above-mentioned antibody. In this invention, "toxin" means various protein, polypeptides, etc. which show the cytotoxicity of a microorganism, an animal, or the vegetable origin. For example, the following can be mentioned as known toxin.
[0071] A diphtheria-toxin A chain (Diphtheria toxin A Chain), (Langone J.J., et al., Methods in Enzymology, 93, 307-308, 1983) The Pseudomonas endotoxin (Pseudomonas Endotoxin), (Nature Medicine, 2, 350-353, 1996) A lysine A chain (Ricin A Chain) Fulton R.J. and et al. -- J.Biol.Chem., 261, and 5314- 5319 and 1986 ; Sivam G., et

al., Cancer Res., 47, 3169-3173, and 1987 ; Cumber A.J. et al. and J.Immunol. Methods, 135, and 15-24, 1990; Wawrzynczak E.J., et al., Cancer Res., 50, 7519-7562, 1990 ; Gheeite V., et al., J.Immunol.Methods, 142, 223-230, 1991 ; [0072] Non-sugar chain lysine A chain () [Deglycosylated] Ricin A Chain () [Thorpe] P. E., et al., and Cancer Res., 47, and 5924-5931, 1987A chain () ; (A Chain) [Wawrzynczak E.J., et al., and Br.J.Cancer 66 361-366, 1992; Wawrzynczak E.J.,] [et] al., Cancer Res., and 50, 7519-7562, 1990 ; Sivam G., et al., and Cancer Res., 47, 3169-3173, and 1987 ; Thorpe P.E. and et al., Cancer Res., 47, and 5924-5931, 1987 ; A gelonin (Gelonin) () [Sivam G., et al.,] [Cancer] Res., 47, and 3169-3173, 1987; Cumber A.J.et al. and J.Immunol.Methods, 135, 15-24, 1990 ; Wawrzynczak E.J., et al., Cancer Res., 50, 7519-7562, 1990 ; Bolognesi A. and et al., Clin.exp.Immunol., 89, 341-346, 1992 ; [0073] Pork WIDO anti-virus protein () [PAP-s] ; Pokeweed anti-viral protein fromseeds (Bolognesi A., et al., Clin.exp.Immunol., 89, 341-346, 1992); BURIOJIN (Briodin) () [Bolognesi] A., et al., and Clin.exp. Immunol., 89, 341-346, 1992 (Bolognesi A., et al., Clin.exp.Immunol., 89, 341-346, 1992) ; Saporin (Saporin); [0074] Peach RUJIN (Momordin) () [Cumber] A. J., et al., and J.Immunol. Methods, 135, and 15-24, 1990; Wawrzynczak E.J., et al. and Cancer Res., 50, 7519-7562, and 1990; Bolognesi A. and et al., Clin.exp.Immunol., 89, 341-346, 1992 (Bolognesi A., et al., Clin.exp.Immunol., 89, 341-346, 1992) ; A MOMORU caulking (Momorcochin) ; Dianthin 32 (Dianthin32) Bolognesi A. and et al. -- Clin.exp.Immunol., 89, 341-346, 1992; dianthin 30 (Dianthin 30) (Stirpe F., Barbieri L., FEBS letter [195], 1-8, 1986); [0075] MODESSHIN (Modeccin) () [Stirpe] F., Barbieri L., and FEBS letter 195, 1-8, and 1986 ; Screw cumin (Viscumin) () [Stirpe] F., Barbieri L., and FEBS letter 195, 1-8, and 1986 ; BORUKESHIN (Volkesin) () [Stirpe] F., Barbieri L., and FEBS letter 195, 1-8, 1986 (Stirpe F., Barbieri L., FEBS letter 195, 1-8, 1986) ; Dodecandrin (Dodecandrin); [0076] TORICHIN (Tritin) () [Stirpe] F., Barbieri L., and FEBS letter 195, 1-8, and 1986; RUFIN (Luffin); (Stirpe F., Barbieri L., FEBS letter 195, 1-8, 1986) A TORIKO giraffe (Trichokirin) (346 Casellas P., et al., and Eur. J. Biochem. 176,581 -588 and 1988; Bolognesi A., et al., and Clin. exp. Immunol., 89, 341- 1992) . [0077] As an antitumor agent Mel FARAN (Melphalan) () [Rowland] G. F., et al., and Nature 255, 487-488, and 1975 ; Cisplatin (Cis-platinum) () [Hurwitz E.and Haimovich] J. and Method In Enzymology 178, 369-375, and 1986 ; Schechter B. and et al., Int.J.Cancer 48 and 167-172, 1991 ; Carboplatin (Carboplatin) ; (Ota, Y., et al., Asia-Oceania J.Obstet.Gynaecol.19, 449-457, 1993) Mitomycin C (Mitomycin C) (it Noguchi(s)) A., et al., Bioconjugate Chem.3, 132-137, 1992; [0078] Adriamycin () [Adriamycin] (Doxorubicin) (it Shih(s)) L. B., et al., and Cancer Res.51 4192-4198 and 1991 ; Zhu, Z., et al., and Cancer Immunol.Immumother40, 257-267, 1995 ; Trail, P. A., et al., and Science 261, 212-215, and 1993 ; Zhu, Z., et al., and Cancer Immunol.Immumother 40,257-267, 1995 ; Kondo, Y. -- et al. -- Jpn.J.Cancer Res.86 1072-1079 and 1995 ; Zhu, Z., et al., and Cancer Immunol.Immumother 40, 257-267, 1995; Zhu, Z., et al., Cancer Immunol.Immumother40, 257-267, 1995 ; [0079] A daunorubicin (Daunorubicin) Dillman and R.O.et al. -- Cancer Res.48 and 6097-6102, 1988; Hudecz, F., and et al. and Bioconjugate Chem. 1, 197-204, 1990; Tukada Y.et al. and J.Natl. Cancer Inst.75 and 721-729, 1984 ; Bleomycin (Bleomycin) Manabe, Y., and et al. -- Biochem.Biophys.Res.Commun.115, 1009-1014, and 1983 neocarzinostatins (Kitamura K. and et al. --) (Neocarzinostatin); Cancer Immunol.Immumother 36, 177-184, 1993 ; Yamaguchi T., et al., Jpn.J.Cancer Res.85, 167-171, 1994 ; [0080] A methotrexate (Methotrexate) Kralovec, J., and et al. -- Cancer Immunol.Immumother 29, 293-302, and 1989 ; Kulkarni, P.N., and et al., Cancer Res.41 and 2700-2706, 1981; Shin, L.B., and et al. and Int.J.Cancer 41, 832-839, 1988 ; Gamett M. C., et al., and Int.J. Cancer 31 and 661-670, 1983 ; 5-fluoro uridine (5-Fluorouridine) () [Shin, L.B.,] [Int.J.] Cancer 46 and 1101-1106, 1990 (Goerlach A., et al., Bioconjugate Chem.2, 96-101, 1991) ; 5-fluoro-2'-deoxyuridine (5-Fluoro-2'-deoxyuridine); [0081] Cytosine arabinoside () [Cytosine] arabinoside () [Hurwitz] E., et al., and J.Med. Chem.28, 137-140, and 1985 ; An aminopterin (Aminopterin) Kanellos J. and et al. -- Immunol.Cell.Biol. 65, 483-493, 1987 (Johnson J.R., et al., and Br.J.Cancer 42, 17, 1980) ; Vincristine (Vincristine); [Vindesine (Vindesine)] (Johnson J.R., et al., and Br.J.Cancer 44, 472-475, 1981) ; etc. is mentioned.

[0082] The disulfide bond for which the connection machine in case the fragment and toxin, or the chemotherapic drug which has an anti-tissue-factor antibody or antigen binding capacity couples directly through the connection machine which these selves have used the sulfhydryl group is mentioned. The disulfide bond in a molecule of Fc field of an antibody is returned in dithiothreitol etc., the disulfide bond of toxin is returned similarly, and both are connected in a disulfide bond. It is Ellman's reagent (Ellman's reagent) before connection. An antibody or toxin is activated and the disulfide bond of both is carried out.

[0083] A connection machine in case the fragment and toxin, or the chemotherapic drug which has an anti-tissue-factor antibody or antigen binding capacity couples directly through the connection machine which these selves have For example, a daunorubicin (Hurwitz, E.et al., Cancer Res.35, 1175-1181, 1975), ad rear MAINSHI (it Mohamed(s) DOKISORUBISHIN; --) G.et al. and Pro.A.A.C.R. 27, 317, 1986 (it Kulkarni(s)) P. N.et al. and Cancer Res.41 and 2700-2706, The 1981 activity ester method (N-hydroxysuccinimide method) activity ester method () [Kulkarni, P.N.et al., Cancer Res.41, 2700-2706,] [1981 Mixed] As a chemotherapic drug connected by anhydride Mixed anhydride Burnstein, S.et al., J.MED.Chem.20, 950-952, and 1977 diazo reactions For example, MTX (De Carvalho, S.et al., Nature (London) 202,255-258, 1964) is used.

[0084] The compound which has [two or more] the amino group, a carboxyl group, a sulfhydryl group, etc. as a connection machine is mentioned, and it is connected by ester combination formed between the carboxyl group or amino acid which the fragment and the toxin, or the enzyme which has an antibody or antigen binding capacity has, the carboxyl group which a linker offers and amino acid, a sulfhydryl group, etc., amide combination, thioester combination, etc. For example, the following matter is used.

[0085] N-SUKUSHINIMIJIRU 3-(2-pyridyl dithio) propionate (SPDP : N-Succinimidyl 3-(2-pyridylditio) propinate) (Wawrzynczak E.J. --) et al. and Cancer Res., 50, 7519-7562, and 1990 ; Thorpe P.E. and et al., Cancer Res., 47, and 5924-5931, 19873-(2-pyridyl dithio) propionate () ; LC-SUKUSHINIMIJIRU (LC-SPDP : LC-Succinimidyl 3-(2-pyridylditio) propinate) [Hermanson] G. T., BIOCONJUGATE Techniques, 230-232, 1996 ; [0086] Sulfo-LC-

SUKUSHINIMIJIRU 3-(2-pyridyl dithio) propionate (Sulfo-LC-SPDP : Sulfo-LC-Succinimidyl 3-(2-pyridylditio) propinate) (Hermanson G.T., BIOCONJUGATE Techniques, 230-232, 1996) 3-(2-pyridyl dithio) butyrate;N-SUKUSHINIMIJIRU () [SPDB:N-Succinimidyl] 3-(2-pyridylditio) butyrate () [Wawrzynczak E.J.,] [et] al. and Br.J.Cancer and 66, 361-366, 1992 ; SUKUSHINIMIJIROKISHI carbonyl-alpha-(2-pyridyl dithio) toluene (SMPT: Succinimidyloxycarbonyl-alpha-(2-pyridylditio) toruene) (Thorpe P.E., et al., Cancer Res., 47, 5924-5931, 1987) ; [0087] LC-SUKUSHINIMIJIROKISHI carbonyl-alpha-(2-pyridyl dithio) toluene (LC-SMPT : LC-Succinimidyloxycarbonyl-alpha-(2-pyridylditio) toruene) ; (Hermanson G.T., BIOCONJUGATE Techniques, 232-235, 1996) Sulfo-LC-SUKUSHINIMIJIROKISHI carbonyl-alpha-(2-pyridyl dithio) toluene () [Sulfo-LC-SMPT] : alpha-(2-pyridylditio) toruene Sulfo-LC-Succinimidyloxycarbonyl- (BIOCONJUGATE Techniques Hermanson G.T. --) 232-235, 1996 ; [0088] SUKUSHINIMIJIRU-4-(p-maleimide phenyl) butyrate (SMPB : Succinimidyl-4-(p-maleimidophenyl) butyrate) () [Hermanson G.T.,] [BIOCONJUGATE] Techniques and 242-243, 1996 ; Sulfo-SUKUSHINIMIJIRU 4-(p-maleimide phenyl) butyrate (Sulfo-SMPB : Sulfo-Succinimidyl-4-(p-maleimidophenyl) butyrate) () [Hermanson] G. T., BIOCONJUGATE Techniques, 242-243 and 1996m-maleimide benzoyl-N-hydro KISHISUKUSHINIMIDO ester () (MBS:m-Maleimidobenzoly-N-hydroxysuccinimide ester); [Hermanson] G. T., BIOCONJUGATE Techniques, 237-238, 1996 ; [0089] Sulfo-m-maleimide benzoyl-N-hydro KISHISUKUSHINIMIDO ester (Sulfo-MBS : Sulfo-m-Maleimidobenzoyl-N-hydroxysuccinimide ester) (Hermanson G.T., BIOCONJUGATE Techniques, 237-238, 1996);S-acetyl mercapto SUKUSHINIKKUANHIDORAIDO () [SAMSA:S-Acetyl mercaptosuccinic] anhydride () [Casellas] P., et al., and Eur.J. Biochem.176 and 581-588, 19883 and 3-dithio screw PURORIONIMI date () ; Dimethyl (DTBP : Dimethyl 3, 3 '-ditiobisprorionimidate) [Casellas P., et al.,] [Eur.] J.Biochem.176, 581-588, 1988 (Thorpe P.E., et al., Cancer Res., 47, 5924-5931, 1987) ; 2-imino thio lane (2-Iminotiolane).

[0090] as a middle base material -- a peptide (PGA), for example, poly L-glutamic acid, a carboxymethyl dextran, a dextran, an amino dextran, an avidin biotin, the cis aconitic acid, glutamic-acid dihydrazide, and human serum albumin (HSA) etc. -- it is used The in TANA rise of the fragment which has an anti-tissue-factor antibody or antigen binding capacity can be carried out to the cell which has discovered the tissue-factor antigen. Therefore, the fragment, a linker, and the various chemotherapic drugs and various toxin that have an anti-tissue-factor antibody or antigen binding capacity and by which middle base material connection was carried out are efficiently introduced into a cell with the internalization of an anti-tissue-factor antibody, and demonstrate those pharmacology effects within a cell.

[0091] Therefore, the complex which connects the fragment and chemotherapic drug which have the anti-tissue-factor antibody or antigen binding capacity of this invention, or toxin, and changes is useful as a medicine constituent for introducing various chemotherapic drugs or various toxin into a cell. Since the tissue factor is widely distributed over the tumor cell, especially the medicine constituent of this invention is useful as a medicine constituent with antitumor action. This effect was proved according to the killer cell effect having been high in the former, when the complex of this invention which changes including the fragment and toxin which have for example, an anti-tissue-factor antibody or antigen binding capacity, for example, immunotoxin, and the toxin of isolation were added into the cell which has discovered the tissue factor.

[0092] The whole body or a partial target can be medicated with the medicine constituent which changes including the complex or this complex which connects the fragment and chemotherapic drug which have the anti-tissue-factor antibody or antigen binding capacity of this invention, or toxin, and changes taking-orally-wise or parenterally. As parenteral medication, the injection in a vein of intravenous drip etc., an intramuscular injection, intraperitoneal injection, and a subcutaneous injection can be chosen, and a medication method can be suitably chosen according to a patient's age and a symptom, for example. The patient already afflicted by the neoplasm is medicated with the medicine constituent which changes including the complex or this complex of this invention in sufficient amount, in order to recover or to prevent a symptom partially at least.

[0093] Moreover, the constituent for a diagnosis which changes including the complex or this complex of this invention is medicated with the localization of the neoplasm of the inside of the body of the patient already afflicted by the neoplasm at a imaging **** sake. For example, the amount of effective medication is chosen in 0.01 to 100mg. [per weight per time of 1kg] Or the 1-1000mg per patient of the amounts of medication of 5-50mg can be chosen preferably. However, the medicine constituent which changes including the complex or this complex of this invention is not restricted to these amounts of medication. Moreover, a medication period can be suitably chosen according to a patient's age and a symptom. The medicine constituent which changes including the complex or this complex of this invention may contain both the support and additives that are permitted in medicine according to a route of administration.

[0094] As an example of such support and an additive, water, the organic solvent permitted in medicine, A collagen, polyvinyl alcohol, a polyvinyl pyrrolidone, a carboxyvinyl polymer, Carboxymethylcellulose sodium, sodium polyacrylate, Sodium-alginate, water-soluble dextran, and carboxy-methyl-starch sodium, Pectin, a methyl cellulose, an ethyl cellulose, xanthan gum, Gum arabic, casein, gelatin, an agar, a diglycerol, a propylene glycol, The surfactant permitted as a polyethylene glycol, vaseline, paraffin, a stearyl alcohol, stearin acid, a human serum albumin (HSA), a mannitol, a sorbitol, a lactose, and a medicine additive is mentioned.

[0095] there is an additive used out of the above suitably according to a pharmaceutical form -- it is -- although combined and chosen, it is not limited to these this invention also includes simultaneous or successive combined use medication with the complex of this invention, other medicines, a biological, a synthetic medicine tablet, etc. again. as other medicines -- anti-inflammation medicine -- anti- -- it is chosen from an allergy medicine, anti-platelet medicine, and other anti-neoplasm medicine

[0096]

[Example] Next, an example explains this invention still more concretely.

the complex of the anti-man tissue-factor monoclonal antibody of the production this invention of the complex (immunotoxin) of an example 1. anti-tissue-factor antibody and a gelonin, and the gelonin (gelonin and Inland Laboratories) which is toxin which has the protein synthesis prevention activity of the vegetable (Gelonium multiflorum) seed origin -- Thrope, P.E.et al., Cancer Res.(1987) 47, and 5924-5931 ** -- it produced using the method It is a control antibody (MOPC-31C) (the complex of ATCC CCL-130 (Yoshida T.H.et al., J.Natl.Cancer Inst.(1968) 41, 1083-1097) and a gelonin was produced.) similarly.

[0097] Sodium phosphate-150mM of 50mM(s) of 1mL NaCl-5mM 2mg of antibodies and it are received in the EDTA (pH 7.0) buffer solution. N-SUKUSHINIMIJIRU of the 3 time molar quantity dissolved in DMSO of 0.01mL(s) immediately before 3-(2-pyridyl dithio) propionate (SPDP and Pierce) is added. After stirring quietly and making it react at a room temperature for 1 hour, It is 50mM sodium phosphate-150mM beforehand. NaCl-5mM By desalting column Fast Desalting Column 10/10 (Pharmacia Biotec) which equilibrated with the EDTA (pH 7.0) buffer solution Unreacted SPDP and unreacted salts were removed and the PDP basis introduction antibody was obtained.

[0098] On the other hand, it is 50mM sodium phosphate-150mM of 1mL. NaCl-5mM The 2-imino thio lane (2-IT and Piece) of 3 time molar quantity is added to gelonin 1mg and it in the EDTA (pH 7.0) buffer solution. After stirring quietly and making it react for 45 minutes at a room temperature, It is 50mM sodium phosphate-150mM beforehand. NaCl-5mM By desalting column Fast Desalting Column 10/10 (Pharmacia Biotech) which equilibrated with the EDTA (pH 7.0) buffer solution Unreacted 2-IT and unreacted salts were removed, and the sulfhydryl group introduction gelonin was obtained. In order to combine an antibody and a gelonin, the sulfhydryl group introduction gelonin was mixed with the PDP basis introduction antibody, and 5000rpm and after carrying out centrifugal concentration for 1 hour and making it about 0.5 mL(s), it put at 4 degrees C by 4 degrees C at Centricon 10 (Amicon) overnight.

[0099] In order to make a reaction end, the iodoacetamide (Nakarai Tesuku) was added so that it might become 0.5% of final concentration at reaction mixture, and it stirred quietly for 30 minutes at the room temperature. In order to remove reagents and an unreacted gelonin, reaction mixture was refined at 4 degrees C, 12000rpm and the supernatant liquid which carried out at-long-intervals heart separation for 10 minutes were refined in gel filtration column Superdex 200 HR 10/30 (Pharmacia Biotech), the fractions containing an antibody and the complex of a gelonin were collected, and it considered as the complex sample of an anti-tissue-factor antibody and a gelonin.

[0100] each sample which is the complex of the complex of the protein synthesis prevention effect anti-tissue-factor antibody and gelonin to the man vesical cancer cell J82 of the complex (immunotoxin) of an example 2. anti-tissue-factor antibody and a gelonin, MOPC-31C, and a gelonin 10% After preparing to 400nM(s) in FCS content RPMI-1640 culture medium and carrying out filtration sterilization with the filter (Millipore) of a 0.22-micrometer aperture, the solution which carried out 3-5 stage dilution of each sample by dilution 10 times was prepared.

[0101] It is 10% fetal calf serum (FCS) about the man vesical cancer cell J82. It suspends in content RPMI-1640 culture medium (Gibco), and is per [2x103] hole to 96 hole cultivation plate (Corning) for tissue culture. It scatters a cell (75microL) and they are 37 degrees C and 5%. CO2 It cultivated under conditions for 6 hours. L addition per [of 25micro of each] 96 hole cultivation plate 1 hole of the diluted solution of the complex sample of an antibody and a gelonin was done. It is 10% after sample addition in the 48th hour. 3-H-leucine diluted 10 times using FCS content RPMI-1640 culture medium (Amersham, 37 MBq/mL, Cat No.TRK636) 10microL addition per hole of was done at 96 hole cultivation plate. 37 degrees C, 5% CO2 The culture medium was removed after carrying out an incubation under conditions for further 20 hours.

[0102] Trypsin/EDTA (sigma, Cat No.T-4049, lot No.3387) 50microL addition per hole of was done. 37 degrees C, 5% CO2 After carrying out an incubation for 5 minutes under conditions, cells were collected to the glass filter (Printed Filter mat A and WALLAC) in the cell harvester (Micro96 HARVESTAR, SKATRON industries company Type No.1057). Measurement of the incorporated 3-H-leucine radioactivity is MicroBeta-1450 (WALLAC, serial No.4500162). It carried out. The protein synthesis prevention activity of antibody-gelonin complex was computed by having made the value of control (it measures only by the culture medium) into 100%. The result was shown in drawing 1 . Consequently, it became clear that an anti-man tissue-factor antibody and gelonin complex show a target-cell unique target the protein synthesis prevention effect.

[0103] the anti-man tissue-factor antibody which has the neutralization activity of the blood coagulation ability of an example 3. tissue factor -- and the killer cell effect man vesical cancer cell J82 to the man vesical cancer cell J82 by the complex (immunotoxin) of an anti-man tissue-factor antibody and a gelonin without neutralization activity -- 10% fetal calf serum (FCS) It suspends in content RPMI-1640 culture medium (Gibco Co.). 2.67x104 The cell suspension prepared to a cell/mL is 75micro[per hole] L Scattered on 96 hole cultivation plate (Corning) for tissue culture, and they are 37 degrees C and 5%CO2. It cultivated under conditions for 6 hours.

[0104] It is each sample of the anti-man tissue-factor antibody-gelonin produced from anti-man tissue-factor monoclonal antibody ATR5, a MOPC-31C-gelonin, and a gelonin 10% The solution which prepared in FCS content RPMI-1640 culture medium, and carried out filtration sterilization with the filter (Millipore) of a 0.22-micrometer aperture was prepared. 25microL addition per 96 hole cultivation plate 1 hole of each manufacture liquid was done, anti-man tissue-factor antibody-gelonin complex and MOPC-31C-gelonin complex were set to 100nM(s), and the gelonin was set to 10microM(s) for final concentration, respectively.

[0105] 71 hours after adding a sample, 20microL addition per hole of Cell Titer 96 Aqueous One Solution Reagent (an MTS reagent and PROMEGA) was done. 37 degrees C, 5% CO2 It is 10% after carrying out an incubation under conditions for 1.5 hours. 25microL addition per hole of a SDS solution was done, the reaction was stopped, and 490-620nm of OD(s) was measured by Microplate reader (Benchmark and Bio Rad). The result was shown in

drawing 2 . Consequently, it became clear that anti-man tissue-factor monoclonal antibody-gelonin complex shows the killer cell effect to a target cell.
[0106] Refining of TF from the refining man placenta of the production 1. man TF of example of reference . anti-TF monoclonal antibody was performed according to Ito's and others method (114 Ito, T. et al. J. Bioc hem. 691 - 696, 1993). That is, it is the degreasing powder which degreased precipitation with the cold acetone after homogenization in the tris buffer physiological salt solution (TBS, pH 7.5) which contains 10mM chlorination BENZAMIJIN, 1mM fluoride [phenylmethyl] sulfonyl, 1mM diisopropyl fluoro phosphate, and 0.02% sodium azide for a man placenta, and was obtained 2% It suspended in the above-mentioned buffer solution containing Triton X-100, and TF was solubilized.
[0107] Concanavalin A-Sepharose 4from this supernatant liquid B Affinity chromatography was performed using the Sepharose 4B column (Pharmacia) which combined the column (Pharmacia) and anti-TF antibody, and Refining TF was obtained. This was condensed by the ultrafiltration membrane (PM-10, Amicon), and it saved at 4 degrees C as a refining preparation. TF content in a refining preparation is commercial anti-TF monoclonal antibody (American Diagnostica). Polyclonal antibody (American Diagnostica) It is combined Sandwich ELISA, and the fixed quantity of the rearranged type TF was carried out and carried out to the standard. Moreover, the purity of a refining preparation was checked by carrying out the argentation color of what [SDS-PAGE / using the 4-20% concentration-gradient polyacrylamide gel / what].
[0108] 2. After mixture with immunity and the Freund's complete adjuvant (Difco) of Freund of dosages, such as the production refining man TF of a hybridoma (about 70mg/(ml)), immunity was carried out to hypodermically [of a 5-weeks old Balb/c system male mouse (Japanese CHARU sliver) / abdomen] so that it might become 10microg / mouse as a TF. TF solution which carried out the booster of the TF mixed with the Freund's incomplete adjuvant of Freund on 12, 18, and the 25th to hypodermically so that it might become 5microg / mouse, and diluted it with PBS as the last immunity on the 32nd was injected intraperitoneally with 5microg / mouse.
[0109] ****** was prepared from four mice three days after the last immunity, and it was made to unite using one fifth of mouse myeloma cell-strain P3U1 and the polyethylene-glycol methods with the number of cells. RPMI-1640 culture medium which contains foetal calf serum for a syncytium 10% (it considers as a RPMI-culture medium below) (Lifetech oriental) It suspended and 400 hole (about 400-piece / hole) seeding was carried out to 96 hole plate per mouse. HAT selection of a hybridoma was performed after fusion by exchanging for the RPMI-culture medium (it considering as a HAT-culture medium below) which will contain HAT (Dainippon Pharmaceutical) and condimed H1 (Boehringer Mannheim GmbH) for the half amount of a culture medium on 1, 2, 3, and the 5th.
[0110] The hybridoma chosen by the following screening procedure was cloned by performing 2 times of limiting dilutions. The limiting dilution carried out seeding of the 0.8 cells per hole to two 96 hole plates. About the hole which has checked that it was a single colony by ****, measurement of TF avidity and TF neutralization activity which were shown below was performed, and the clone was chosen. Habituation of the obtained clone was carried out to the RPMI-culture medium from the HAT medium, after checking that there was no fall of the antibody-production ability by habituation, the limiting dilution was performed again and perfect cloning was performed. The hybridoma which produces six sorts (ATR-2, 3, 4, 5, 7 and 8) of antibodies which check strongly combination with TF / factor VIIa complex, and Factor X by the above operation has been established.
[0111] 3. Production of the ascites of a hybridoma in which production of ascites and the antibody carried out refining establishment was performed according to the conventional method. Namely, hybridoma 106 which carried out the passage by in vitro The individual was beforehand transplanted in the peritoneal cavity of the Balb/c system male mouse which prescribed straight mineral oil for the patient into 2 times peritoneal cavity. Ascites was collected from the mouse with which the abdomen got fat in the 1-2nd week after the transplant. Refining of the antibody from ascites is Protein A. ConSepLC100 equipped with the column (NGK Insulators) System (Millipore) It carried out by using.
[0112]
[Layout Table]
SEQUENCE-LISTING<110> CHUGAI-SEIYAKU-KABUSHIKI KAISHA <120> Complex of-anti-tissue-factor antibody<130> 994674<160> 1 <210> 1 <211> 295 <212> PRT <213> Homosapiens <220> <223> Amino acid sequence of human tissue factor<400> 1 <400> 2 Met Glu Thr-ProAla Trp Pro Arg Val Pro Arg Pro Glu Thr Ala Val -30 - 25 - 20 Ala Arg Thr Leu Leu Leu Gly Trp Val Phe Ala Gln Val Ala Gly Ala - 15 -10 - 5-1 Ser Gly Thr Thr Asn Thr Val Ala Ala Tyr Asn Leu Thr Trp Lys Ser 1 5 10 15 Thr Asn Phe Lys Thr Ile Leu Glu Trp Glu Pro Lys Pro Val Asn Gln 20 25 30 Val Tyr ThrVal Gln Ile Ser Thr Lys Ser Gly Asp Trp Lys Ser Lys 35 40 45 Cys Phe Tyr Thr Thr Asp Thr GluCys Asp Leu Thr Asp Glu Ile Val 50 55 60 Lys Asp ValLys Gln Thr Tyr Leu Ala Arg Val Phe Ser Tyr Pro Ala 65 70 75 80 Gly Asn Val Glu Ser Thr GlySer Ala Gly Glu Pro Leu Tyr Glu Asn 85 90 95 Ser Pro Glu Phe Thr Pro Tyr Leu GluThr Asn Leu Gly Gln Pro Thr 100 105 110 Ile Gln Ser Phe Glu Gln Val GlyThr Lys Val Asn Val Thr Val Glu 115 120 125 Asp Glu Arg Thr Leu Val Arg Arg Asn Asn Thr Phe Leu Ser Leu Arg 130 135 140 Asp Val Phe Gly Lys Asp Leu Ile Tyr Thr Leu Tyr Tyr Trp Lys Ser 145 150 155 160 Ser Ser Ser Gly Lys Lys Thr Ala Lys Thr Asn Thr Asn Glu Phe Leu 165 170 175 Ile Asp Val Asp Lys Gly Glu Asn Tyr Cys Phe Ser Val Gln Ala Val 180 185 190 Ile Pro Ser Arg Thr Val Asn Arg Lys Ser Thr Asp Ser Pro Val Glu 195 200 205 Cys MET Gly Gln Glu Lys Gly Glu Phe Arg Glu Ile Phe Tyr Ile Ile 210 215 220 Gly Ala Val Val Phe Val Val Ile Ile Leu Val Ile Ile Leu Ala Ile 225 230 235 240 Ser Leu His Lys Cys Arg Lys Ala Gly Val Gly Gln Ser Trp Lys Glu 245 250 255 Asn Ser Pro Leu Asn Val Ser 260

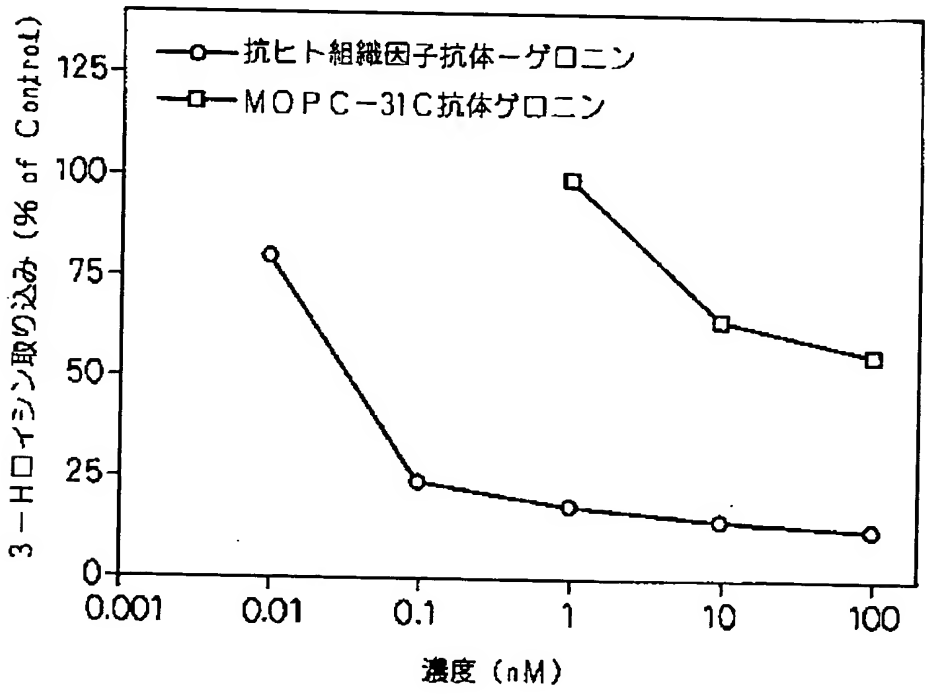
DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] Drawing 1 is by the Homo sapiens vesical cancer origin cell J82 of the anti-human-tissue factor antibody-gelonin complex of this invention, and the MOPC-31C antibody-gelonin complex as contrast. It is the graph which compares and shows the depressor effect (protein synthesis depressor effect) to the incorporation of a 3H-leucine.
[Drawing 2] drawing 2 is the graph which compared the killer cell effect over complex with an anti-human-tissue factor antibody gelonin, the complex of MOPC-31C as contrast, and a gelonin and gelonin independence, and J82 cell boiled and depended

DRAWINGS

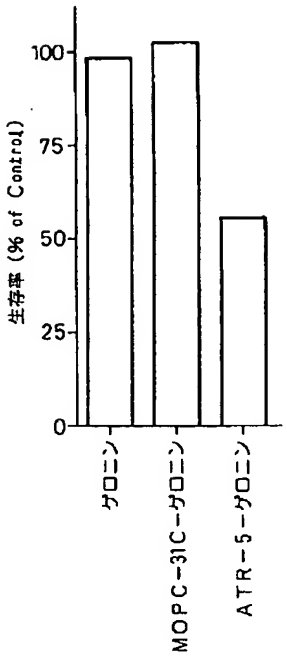
[Drawing 1]

図1



[Drawing 2]

図 2



[Translation done.]